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ORIGINAL ARTICLE

The regenerative role of adipose-derived stem cells (ADSC) in plastic and reconstructive surgery*

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Abstract

The potential use of stem cell-based therapies for the repair and regeneration of various tissues and organs offers a paradigm shift in plastic and reconstructive surgery. The use of either embryonic stem cells (ESC) or induced pluripotent stem cells (iPSC) in clinical situations is limited because of regulations and ethical considerations even though these cells are theoretically highly beneficial. Adult mesenchymal stem cells appear to be an ideal stem cell population for practical regenerative medicine. Among these cells, adipose-derived stem cells (ADSC) have the potential to differentiate the mesenchymal, ectodermal and endodermal lineages and are easy to harvest. Additionally, adipose tissue yields a high number of ADSC per volume of tissue. Based on this background knowledge, the purpose of this review is to summarise and describe the proliferation and differentiation capacities of ADSC together with current preclinical data regarding the use of ADSC as regenerative tools in plastic and reconstructive surgery.

Introduction

In recent years, worthwhile advances in the field of plastic and reconstructive surgery have been achieved in both basic science and clinical research with subsequent translation into patient care. Plastic surgery aims to restore form and function following a wide range of congenital or acquired defects, with procedures often transcending the anatomical boundaries that limit other specialties. With the recent advances in medical imaging, microsurgery, composite tissue allotransplantation, nanotechnology, cell biology and biomaterials, treatment options for patients are wider than ever. For centuries, the 'reconstructive ladder' was restricted to local flaps and skin grafts, and with the advent of microsurgery, the reconstructive armamentarium was vastly expanded. These autologous options are reliable and are generally functionally and aesthetically acceptable. However, many plastic surgeons have become increasingly cognizant that there is the real potential for a paradigm shift in reconstructive surgery. The worldwide surgical community is becoming increasingly aware of the research landscape. Tissue engineering, also known by the term regenerative medicine, is a modern, interdisciplinary field combining principles of both engineering and the life sciences. It shares a common objective with plastic

Key Message

- ADSCs have the ability to produce a range of tissue types and are an ideal cell source for the clinical application of tissue engineering

*This work is attributed to: Reconstructive Surgery Regenerative Medicine Group, Institute of Life Sciences (ILS), Swansea University Medical School, Swansea, UK.

and reconstructive surgery, namely to maintain or restore tissue function.

Tissue engineering offers like-for-like reconstruction without donor site morbidity or immunosuppression by combining stem cell therapy with 3D scaffolds made from biological or synthetic biomaterials and growth factors for custom-designed regenerative solutions. Significant progress has recently been made in the application of mesenchymal stem cells in soft tissue reconstruction and cutaneous wound healing based on the inherent capacity of these cells to enhance angiogenesis, minimise inflammation (1), self-renew and differentiate to specialised cell types under specific physiological conditions (2–4). Autologous adult stem cells have been the predominant cells used as they are immunocompatible, and their use has few ethical concerns as long as informed consent is obtained prior to harvest. This is in contrast to embryonic (ESC), umbilical cord mesenchymal (UCMSC) and induced pluripotent stem cells (iPSC), which have limited clinical use because of problems with cellular regulation and teratoma formation, ethical considerations, immunogenicity (ESC), genetic manipulation (iPSC) and difficulties with long-term storage (UCMSC) (5,6).

Adult mesenchymal stem cells (MSC) are non-haematopoietic cells of mesodermal derivation that are present in a number of organs and connective tissues, including adipose tissue (7–14). Adipose tissue is the predominant source of these cells used clinically and experimentally because significant quantities of adipose tissue can be removed with minimal morbidity. While other tissues can be used, their biopsy volume is more limited without causing morbidity, and therefore, the lower yield of cells requires in vitro expansion before clinical use (2). Since their first description in 2002 (15), there has been significant interest in the potential offered by autologous adipose-derived stem cell (ADSC) (Figure 1) to bridge the translational gap. A number of preclinical and clinical trials have established the safety and efficacy of ADSC (16,17) and range from breast reconstruction and correction of defects (18) to neural regeneration in spinal cord injuries (19). In light of this recent progress in translational ADSC research, it is time to review recent progress in the application of (ADSC) both to reconstruct soft tissue defects and in the enhancement of cutaneous wound healing and determine the remaining challenges to the widespread clinical application of this approach. The Celution system (Cytori Therapeutics, San Diego, CA), currently approved for clinical use, utilises adipose tissue to yield an ADSC-enriched cell mixture. Among other applications, this mixture has been shown to be a valuable method for the treatment of chronic ulcers in lower limbs of arteriopathic patients (20).

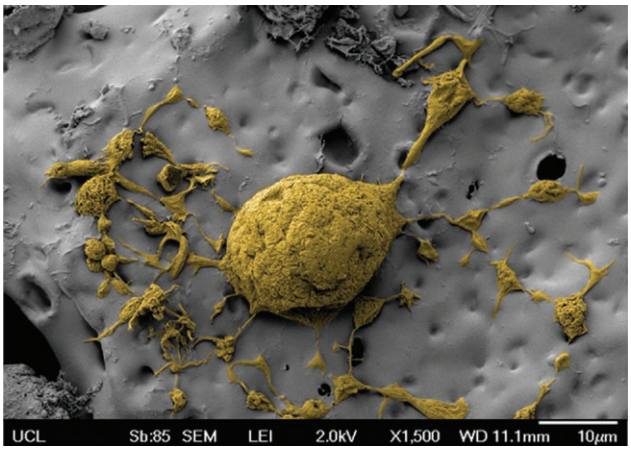

Figure 1 Scanning electron microscopy image of adipose-derived stem cells (ADSCs) on a polyhedral oligomeric silsesquioxane (POSS)-modified poly(caprolactone urea-urethane) (POSS-PCL) nanocomposite polymer scaffold.

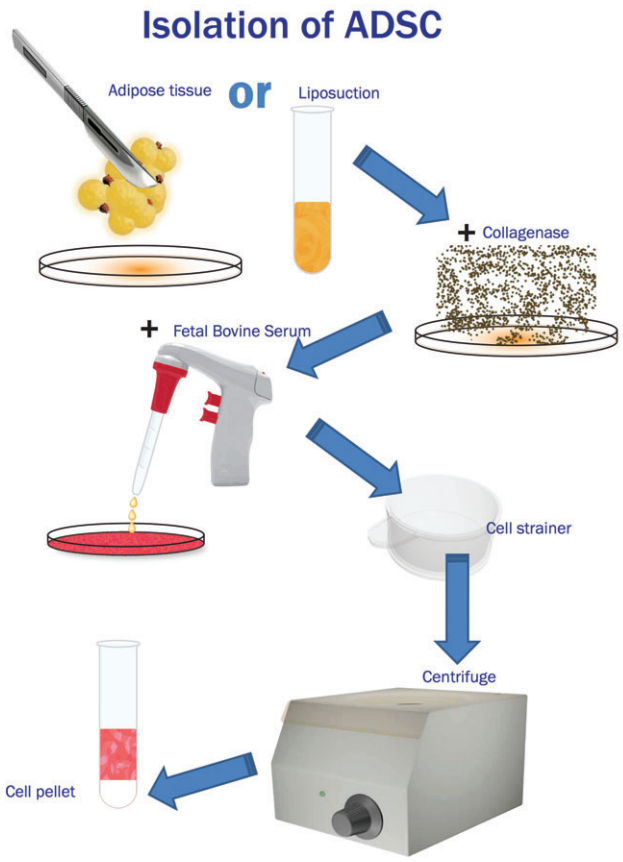

Figure 2 Schematic representation of adipose-derived stem cell (ADSC) isolation from adipose tissue and/or lipoaspirate. After enzymatic digestion, the effect of the enzyme is reversed by foetal bovine serum (FBS), and the mixture is filtered through a cell strainer. The cell pellet remains after centrifuging the mixture and discarding the supernatant.

Adipose-derived stem cells: definition, preparation and application

Whilst ADSCs share many of the characteristics of bone marrow-derived mesenchymal stem cells (BMSC) (21–24), they can be obtained more easily with a 100–1000 times greater cellular yield (25), and in practice, many patients are eager for the harvest of unwanted fat. ADSCs are isolated from the stromal vascular fraction (SVF), which is obtained from adipose tissue by enzymatic digestion (Figure 2). The SVF shows a heterogeneous immunophenotype based on flow cytometry with additional cell types including lymphocytes, endothelial

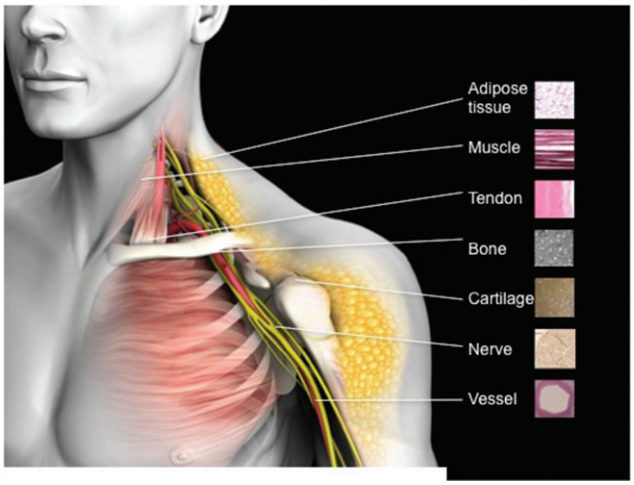

Figure 3 Adipose-derived stem cells (ADSCs) have the potential to differentiate along several lineages and into adipocytes, chondrocytes, osteoblasts, neurons, Schwann cells, vascular endothelial cells and tenogenic cells among other cell and tissue types.

cells, fibroblasts, macrophages, pericytes and preadipocytes (26–28). Subsequent cultures of SVF cells on a tissue culture surface yield an adherent population; these ADSCs are more homogeneous based on their surface immunophenotype (2,26,27,29). Although ADSCs are of mesodermal origin, they can also be differentiated under the correct conditions into cells of ectodermal or endodermal origin. Thus, ADSCs have the potential to differentiate several lineages and into adipocytes, chondrocytes, osteoblasts, neurons, Schwann cells, vascular endothelial cells, tenogenic cells and others, both in vitro and in vivo (2,30–35) (Figure 3).

Both ADSCs and SVF cells display regenerative capacity when applied to a range of animal models of human disease (36), and multiple mechanisms have been proposed to explain their properties. Initial studies suggested that ADSCs act by differentiating along a mesenchymal lineage, thereby helping to replace defective or ablated cells in vivo (37–39). ADSCs may also act through the paracrine release of growth factors required to accelerate and direct tissue repair by host-derived cells (40,41). The introduction of ADSCs into an ischaemic area may, for example, result in their secretion of vascular endothelial growth factor (VEGF), leading to the increased recruitment of local endothelial cells and angiogenesis. The secretion of immunomodulatory factors, such as prostaglandin E2, by ADSCs may suppress host inflammatory responses following an ischaemic event and thereby enhance recovery (26,42,43). Secretion of VEGF, hepatocyte growth factor, FGF-2, and insulin-like growth factor 1 (IGF-1) may enhance angiogenesis and tissue regeneration to the benefit of wound healing. This is of particular interest in challenging wounds such as burn or radiation injuries (44,45). The use of platelet-rich plasma (PRP) in combination with ADSCs has seen great popularity recently. PRP in association with insulin greatly potentiates adipogenesis in human ADSCs through a FGFR-1- and ErbB2-regulated Akt mechanism and ameliorates clinical fat graft maintenance, showing promising results for the translational applications of combined PRP–insulin treatment in regenerative medicine (46).

Phenotypical and functional heterogeneity of adipose-derived stem cells

Heterogeneity, both donor-to-donor and within ADSC populations, can make assessments of their utility, challenging and predicting how culture expansion and external factors such as donor age, method and site of harvest influence the properties of the harvested ADSCs (47–54). This may be because of variability in the rate of proliferation as illustrated by Kalbermatten *et al.* (47) who isolated cells from the superficial and deep layers of abdominal adipose tissue and found that cells isolated superficially proliferated more quickly. In animal models, a study focusing on the use of ADSCs in nerve regeneration found that after culture with specific growth factors, tissue harvested from the neck and flank region showed the highest levels of neural-specific proteins (49). Oedayrajsingh-Varma *et al.* (48) proposed that while adipose tissue harvested from the hips and abdomen* was suitable for tissue engineering, the presence of glandular cells in tissue harvested from the breasts could cause potentially unacceptable variability in cell counts. The cellular function may be adversely affected by surgical technique, with a number of studies suggesting that an atraumatic harvest† versus traditional liposuction may result in a healthier cell population that is more capable of surviving transplantation (50–52). The angiogenic potential of cells has also been shown to decline with advancing donor age, caused by a reduction in the quantities of pro-angiogenic factors being produced (55).

Regenerative applications of ADSCs

Conventional vascularised tissue transfer is the mainstay of current tissue reconstruction, but it produces donor-site morbidity, and not all defects can be reconstructed adequately with this approach. While composite vascularised tissue allotransplantation (CTA) is an emerging field used for the reconstruction of complex tissues such as hands and the face, the replacement of the whole structure as a unit is usually necessary, and life-long immune suppression is still mandatory for these patients. The use of adult stem cells for cell-based tissue engineering and regeneration strategies represents a promising approach for the repair of many critical tissues, including vasculature, muscle, nerves, cartilage and skin. Tissues that are not currently reconstructible, such as cardiac muscle or central nervous tissue, which transforms into non-functional fibrous tissue following infarction, would be ideal for this approach and reduce the need for organ or tissue transplantation as a result. For this approach to be successful, the aetiology of the tissue deficit must be addressed. For example, a patient with a systemic myopathy may be unsuitable for this approach, but studies of the regenerative capacities of stem cells in this setting may provide new insights into the treatment of these types of conditions.

*Harvested by either tumescent liposuction or resection.
†Coleman technique or cannula and syringe.

Regeneration and angiogenesis

Several studies suggest that ADSCs have the potential to differentiate into endothelial cells, secrete paracrine factors that stimulate endothelial repair and prevent neointimal formation. As a result of their angiogenic properties, ADSCs have novel therapeutic potential in a wide range of ischaemic conditions, such as myocardial infarctions, reno-vascular, peripheral vascular and cerebrovascular diseases. Studies examining the effects of ADSCs in renal artery stenosis (RAS) found the suppression of inflammatory cytokines and contribution to neovascularisation (56) through the initial paracrine delivery of factors such as VEGF (57). Similarly, during in vivo testing of ischaemic animal tissue, ADSCs were found to promote new vessel formation and angiogenesis along with vascular remodelling (58). Functional improvements associated with these vascular changes were also demonstrated, with ADSCs having a positive effect on cardiac remodelling with a decrease in fibrotic change and cardiac hypertrophy in the months following myocardial infarction (59,60).

Neural regeneration

Peripheral

Neural tissue exemplifies the paradox between the use of the most suitable cell for neural regeneration (e.g., the Schwann cell) and the donor deficit in harvesting autologous Schwann cells for this approach. Schwann cell (SC) transplantation has been shown to enhance peripheral nerve repair, but the clinical application of this technique is limited by donor-site morbidity and the inability to generate sufficient numbers of SC quickly (61). Instead, incorporation of ADSCs into nerve conduits has been shown to enhance neural regeneration similar to autograft transplantation by offering a more nerve-like environment and releasing several neurotrophic factors (62–64) (Table 1). It has been well reported that better functional and histological results can be obtained by differentiating the ADSCs into an SC-like phenotype prior to transplantation (62,65). However, further investigation of the long-term ADSC survival and functionality combined with nerve conduits in peripheral nerve repair is required.

Central

ADSCs have a significant role in tissue repair by virtue of their ability to home to the injured central nervous system coupled with their ability to suppress inflammation. This property may have therapeutic benefit in autoimmune diseases such as multiple sclerosis and encephalomyelitis (66–69), and research into these areas is on-going.

Adipose regeneration

Several studies suggest that ADSCs are highly effective in forming de novo adipose tissue (49,70–74) (Table 2, Figure 4) with evidence supporting the use of collagen scaffolds to provide support during the process of neo-adipogenesis (56,59). The paracrine mechanism of ADSCs promotes the secretion of several growth factors, potentially enhancing their ability to regenerate adipose tissue (75). Adipose tissue regeneration has been shown to benefit breast reconstruction by enhancing volume and improving cosmesis and breast symmetry (15,31,76). Lipofilling is a minimally invasive technique transplanting autologous cells, which, in addition to creating volume, also provides a complementary effect within the receptor zone (77). The small population of stem cells within the fat graft may contribute to the improved remodelling through the production of signalling molecules and growth factors (66). These techniques are particularly useful and increasingly employed following breast-conserving surgery for breast cancer. In this context, however, we must consider the possibility that autologously grafted MSCs might contribute to carcinogenesis and invasive potential by autocrine and paracrine effects (78,79). A number of studies are currently examining the local and systemic effects of ADSCs on breast cancer cells (80–83).

Tendon regeneration

The goal of primary tendon repair is to restore tensile strength at the time of mobilisation. Current repair strategies using primary repair, suitable autografts and freeze-dried allografts lead to a slow repair process that is sub-optimal and often fails to restore function completely (84). A repaired tendon never completely regains the biological and mechanical properties of an undamaged tendon; the collagen fibrils remain thinner, and the tendon's mechanical strength is reduced (85). Tendon regeneration using MSCs has been described in several studies; however, the use of ADSCs for tendon tissue engineering has only recently been considered (Table 3). Local administration of ADSCs to the site of injury appears to accelerate tendon repair (86), as exhibited by a significant increase in tensile strength, direct differentiation of ADSCs toward tenocytes and endothelial cells and increases in angiogenic growth factors (87).

Three-dimensional scaffolds have been shown to support the proliferation and adhesion of ADSCs, which, under the influence of growth differentiation factor-5 (GDF-5), have successfully produced type 1 collagen (84). However, while a number of studies have shown the potential for the use of ADSCs in tendon repair and identified that GDF-5 may have a significant role to play in tendonogenic differentiation, further studies are needed to determine the ideal concentration of growth factors and conditions required for effective tendon repair (2,24,84,87,88).

Cartilage regeneration

In our aging populations, the high rates of arthritis impact joint mobility and quality of life (Table 4). As a result of this massive market, there has been intense interest in the use of stem cell-based therapies for articular cartilage regeneration. The current treatments for articular cartilage injury revolve primarily around symptom control, having little effect on disease progression (89). There is a lack of inherent mechanisms for the regeneration of mature articular cartilage, and surgical options for repair or replacement generally result in the formation of fibrocartilage rather than hyaline (90–93). After initial experiments demonstrating the potential for regeneration, MSCs under the right conditions have since been shown to

Table 1 Nerve tissue regeneration with ADSCs

Year (reference)	ADSC	In vivo/in vitro	Scaffold	Growth factors	Differentiation	Tissue regeneration
2013 (130)	Dog	Dog	PTFE conduit	–	–	Facial nerve regeneration
2013 (131)	Rat	In vitro	TCP, laminin/ fibronectin coated TCP	Coculture with various neural cells	–	Enhanced NTF release by SC-like cells
2013 (132)	Human	Rat	NGF-hydrogel	NGF within hydrogel	–	Erectile function improvement
2012 (133)	Human	In vitro	–	BHA, RA, EGF, and bFGF	Neuron-like cells	PNR in vivo
2012 (134)	Rat	In vitro	–	DM	–	PNR in vitro
2012 (135)	Rat	Rat	GGT*	DM	Neuro-like cells	PNR in vivo
2012 (136)	Rat	Rat	Silicon	DM	SC	PNR in vivo
2012 (62)	Rat	Rat	DNA	DM	SC-like cells	PNR in vivo
2012 (137)	Rat	In vitro	–	N2, ABAM, B27, EGF, and bFGF	Glial-like cells	PNR in vitro
2012 (138)	Rat	Rat	GGT	–	–	PNR in vivo
2011 (139)	Rat	Rat	PCL	DM	SC	Prevention of DRG neuronal loss
2011 (140)	Rat	Rat	Allogeneic artery	DM	SC	PNR and functional recovery in vivo
2011 (141)	Rat	Rat	ANAs	–	–	PNR in vivo
2011 (142)	Rat	In vitro	–	DM	SC-like cells	–
2011 (143)	Rat	Rat	Chitosan/ silk fibroin	–	–	PNR in vivo
2011 (144)	Human	In vitro	–	BHA, RA, EGF, bFGF	Neuron-like cells	Neuronal differentiation
2011 (145)	Rat	Rat	ADMT	–	–	Cavernous nerve in vivo
2011 (146)	Rat	Rat	Fibrin	DM	SC-like cells	PNR in vivo
2011 (147)	Rat	In vitro	–	DM	SC-like cells	ADSC from SC and PN fat were more effective†
2011 (148)	Mice	Mice	Matrigel	–	–	NTF release
2011 (149)	Rat	Rat	Vein graft	–	–	PNR in vivo
2010 (150)	Rat	Rat	PHB sheet	–	–	PNR in vivo
2010 (151)	Rat	In vitro	PCL and PLLA	DM	SC	PNR in vitro
2010 (152)	Rat	Rat	Fibrin conduit	DM	SC-like cells	PNR in vivo
2010 (153)	Rat	Rat	XANM	DM	SC	PNR in vivo
2010 (154)	Rat	Rat	–	DM	SC	CNR in vivo
2009 (155)	Rat	In vitro	–	DM	SC-like cells	–
2009 (156)	Rat	In vitro	–	DM	Glial cell	NTF release
2009 (157)	Human	Nude rat	PCL	–	–	PNR in vivo
2008 (158)	Rat	In vitro	–	DM	SC-like cells	Myelin formation
2007 (159)	Rat	In vitro	–	DM and forskolin	SC-like morphology	PNR in vivo

ADMT, adipose tissue-derived acellular matrix thread; ANA, acellular nerve allografts; bFGF, basic fibroblast growth factor; BHA, butylated hydroxyanisole; CNR, Central nerve tissue regeneration; DM, differentiation medium containing PDGF, bFGF and GGF-2; DNA, decellularized nerve allografts; EGF, epidermal growth factor; NGF, nerve growth factor; NTF, neurotrophic factor; PCL, poly-ε caprolactone; PHB, poly-3-hydroxybutyrate; PLLA, poly-D-L-lactic acid; PN, perinephric; PNR, peripheral nerve tissue regeneration; PTFE,, polytetrafluoroethylene; RA, retinoic acid; SC: subcutaneous. TCP, tissue culture plastic; XANM: Xenogenic acellular nerve matrix.

*GGT nerve conduit containing genipin crosslinked gelatin annexed with tricalcium phosphate.

†Compared to epididymal fat in Wistar rats.

differentiate into chondrocytes (77,94–97) (Figure 4). There are a number of growth factors that affect the repair of cartilage and the differentiation of MSCs to chondrocytes; in vitro studies examining growth factors have shown that TGFβ and FGF-2 induces the proliferation of cells (92,96,98–100) and promotes the chondrogenic differentiation of MSCs (101,102). While studies examining ADSCs have demonstrated the successful production of cartilage in vivo (95), the focus of many studies remains on the use of stem cells derived from bone marrow (BM), which has been shown to produce more architecturally stable cartilage (95,101,103). In addition, this has implications for nasal and auricular cartilage engineering, potentially negating the need for donor-site morbidity and multiple stage operations to achieve a cosmetically desirable result.

Muscle regeneration

Skeletal muscle

Satellite cells are generally regarded as the myocyte precursor within adult tissues. Regeneration of adult skeletal muscle

Table 2 Adipose tissue regeneration with ADSC

Year (reference)	ADSC	In vivo/in vitro	Scaffold	Growth factors	Differentiation	Tissue regeneration
2013 (127)	Human	Both – nude mice	Collagen	–	Adipocytes endothelial cells	Adipose and loose connective tissues in vivo
2013 (160)*	Human and rat	Both – rats	DAT	–	Adipocytes	Adipose tissue and angiogenesis in vivo
2013 (71)	–	Rabbit	PPP mesh, collagen	–	–	Adipose tissue in vivo
2012 (161)	Human	In vitro	–	–	Adipocytes	–
2012 (162)	Human	Both – rats	Cross–linked DAT	–	Adipocytes	Adipose tissue and angiogenesis in vivo
2012 (163)	Porcine	In vivo pigs	Collagen	–	–	Increased thickness connective tissue
2012 (164)	Human	In vivo nude mice	Collagen/gelatin	bFGF	Adipocytes	Increased adipogenesis and angiogenesis with 1 mg/cm^2 bFGF
2011 (165)	Human	In vitro	Silk	VEGF/laminin	Adipocytes	–
2010 (31)	Mice	In vitro	–	–	Adipocytes	–
2010 (72)	Mice	In vivo athymic mice	Collagen type I, PGA, HA	–	Adipocytes	Collagen regenerated more adipose tissue than two other scaffolds
2008 (166)	Human	In vitro	–	–	Adipocytes	Adipogenesis with collagen fibres and vessels at 4 months
2008 (167)	GFP Mice	Both – athymic mice	Fibrin glue	–	Adipocytes	Adipogenesis
2008 (168)	Human	Both – athymic mice	Gelatin, PGA in PPP mesh	–	Adipocytes	Adipose tissue in vivo, preservation of scaffold shape at 6 months

ADSC, adipose-derived stem cell; DAT, decellularised adipose tissue; HA, hyaluronic acid; PGA, polyglycolic acid; PPP: polypropylene.
*In vitro human ADSC differentiation and in vivo rat ADSC implantation.

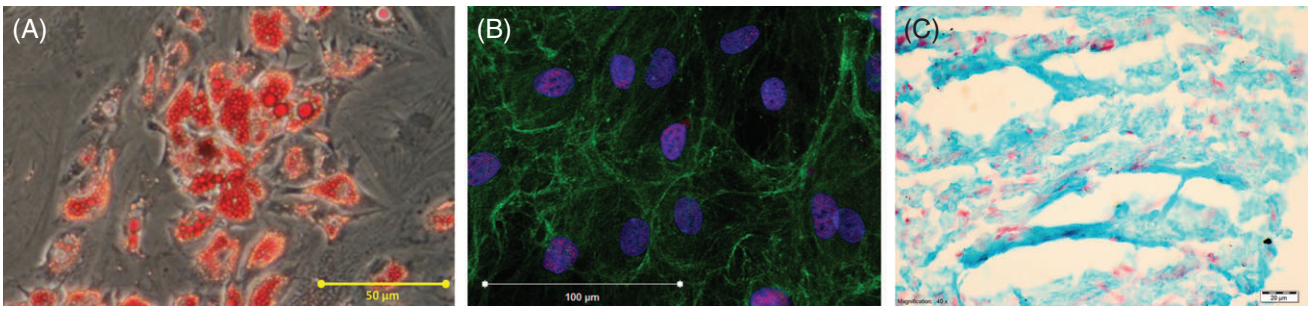

Figure 4 Adipose-derived stem cells (ADSCs) differentiated into adipocytes – Oil Red O (red) stained (A), osteoblasts – RunX2 (red), collagen I (green) and Dapi (blue) stained (B) and chondrocytes – alcian blue (blue) and neutral red (red) stained (C).

occurs when this normally dormant and non-proliferative population of mononucleated myocyte precursors are activated following injury (104). However, the number of these satellite cells within mature muscle represents only 1–5% of the total cell number (105), and their potential to self-renew decreases with age and diseases that cause a gradual and continuous degeneration of the muscle fibres, such as Duchenne muscular dystrophy (DMD) (106). ADSCs have been shown to participate in myofibre formation when exposed to a regenerating muscle environment (2,107) and may additionally contribute to muscle regeneration by modifying gene expression post fusion with the host cells (108).

Smooth muscle

Smooth muscle is a major component of, and essential for, the normal function of cardiovascular, gastrointestinal, reproductive and urinary systems. In the field of cellular therapeutics, one major limitation is a reliable source of smooth muscle cells (SMC) as biopsies of organs containing these cells can be impractical and have associated morbidity. The ease of harvest and low donor-site morbidity of ADSCs make them ideal for use, and their ability to differentiate along multiple cell lineages has been well established (1,2,15). Studies have shown that these cells are capable of inducing the expression of

Table 3 Tendon tissue regeneration with ADSC

Year (reference)	ADSC	In vivo/in vitro	Scaffold	Growth factors	Differentiation	Tissue regeneration
2012 (86)	Rabbit	In vivo rabbit Achilles tendon injury	–	PRP with or without ADSC	Tenocytes	ADSC improved primary tendon healing, increased collagen type I, VEGF and FGF production and decreased TGF-β1, 2, 3 levels
2011 (84)	Rat	In vitro	PLAGA 3D fibre scaffold compared to 2D sheet	GDF-5	Tenocytes	Increased collagen type I gene expression with GDF-5 stimulation of ADSC on 3D scaffolds
2011 (87)	Rabbit	In vivo rabbit Achilles tendon injury	–	PRP with or without ADSC	Tenocytes and endothelial cells	ADSC increase tensile strength, differentiate toward tenocytes and endothelial cells, and increases angiogenic growth factors.
2010 (88)	Rat	In vitro	–	GDF-5	Tenocytes	GDF-5 induces tenogenic differentiation of ADSC.

FGF, fibroblast growth fator; GDF-5, growth differentiation factor-5; PLAGA, poly(dl-lactide-co-glycolide); PRP, platelet-rich plasma; VEGF, vascular endotelial growth factor; TGF-β, transforming growth factor beta.

Table 4 ADSC in cartilage tissue regeneration

Year (reference)	ADSC	In vivo/in vitro	Scaffold	Growth factors	Differentiation	Tissue regeneration
2010 (92)	Rabbit	In vivo Rabit articular cartilage	Gellan Gum Hydrogels	TGF-β1 BMP-2	Chondrocytes	ADSC combined with TGF-β1 & BMP-2 showed up-regulation of type II collagen and aggrecan
2010 (97)	Human	Both – nude mice	Fibrin glue	–	Chondrocytes	Concentration of GAGs increased over time, ADSC differentiated and formed new cartilage
2008 (96)	Human	In vitro	–	TGF-β2 BMP-2,6,7	Chondrocytes	Combination of TGF-β2 and BMP-7 enhance chondrogenesis
2008 (100)	Human	Both – nude mice	PLGA	TGF-β1	Chondrocytes	ADSC-seeded PLGA scaffolds expressed a stable chondrogenic phenotype

ADSC, adipose-derived stem cell; BMP, bone morphogenetic protein; GAGs, glycosaminoglycans; GF, growth factor; PLGA, poly(lactic-co-glycolic acid); TGF-β, transforming growth factor beta.

a smooth muscle phenotype and respond as SMCs to common pharmacological agents (109).

Wound healing and skin regeneration

Wound repair is a complex process of inflammation, angiogenesis, formation of new tissue and finally remodelling (110,111). Stem cells are thought to integrate themselves with the local environment, contributing to wound healing by secreting signalling molecules, accelerating repair and differentiating into the required cells, encouraging similar differentiation within adjacent cells (112). The paracrine effect of ADSCs results from the secretion of cytokines such as TGF-β, VEGF, granulocyte/macrophage colony-stimulating factor (GM-CSF), hepatocyte growth factor (HGF) and stromal derived factor 1 (SDF-1) (40,113,114). In addition to facilitating wound healing, they can also recruit and stimulate endogenous stem cells to participate in the process (105).

The use of progenitor cells in wound healing has been extensively investigated (Table 5) and the use of ADSCs, as previously discussed, has a number of advantages regarding ease of harvest and low rate of donor-site complications (115–117). The autologous transplantation of ADSCs has been shown to increase the survival of full-thickness skin grafts and promote wound healing (118). In recent human studies, there was an improvement reported in the skin quality overlying fat injection sites (119), scar reduction in patients with severe burns (120) and marked improvement in severe radiodermatitis sustained post cancer treatment (121). Delivery systems have also been explored with dermal polymer scaffolds in development to act as a skin substitute to allow optimal stem cell interface with the wound to encourage healing (122).

Multiple groups worldwide are investigating tissue-engineering approaches that combine ADSCs with fibroblasts or other progenitor cells and seeding onto scaffolds to 'manufacture' implantable constructs (123). Various materials

Table 5 Wound healing and skin regeneration with the aid of ADSCs

Year (reference)	ADSC	In vivo/in vitro	Scaffold	Growth factors	Differentiation	Tissue regeneration
2013 (111)	Rabbit	Both – Rabbit	–	–	–	ADSC increase endothelial cell recruitment and enhance wound repair
2012 (128)	Human	In vitro	–	BGFG, EGF	Keratinocytes	Formation of epidermal layer and expression of Keratin 10. Detection of Desmosomes and hemidesmosomes
2012 (129)	Human	In vitro	–	–	Keratinocytes	ADSC protein extracts improved human keratinocyte proliferation and migration
2011 (126)	Mice	Both – GFP transgenic mice	Co-CS-HA and ADM	–	–	ADSC-seeded scaffolds enhanced angiogenesis and wound healing
2011 (118)	Sprague–Dawley rats	Both – rat	–	–	–	ADSC under skin grafts increased angiogenesis and improved wound healing
2009 (124)	Human	Both – athymic mice	Silk fibroin-chitosan	–	Epithelial, endothelial & fibrovascular	ADSC seeded scaffolds enhance wound healing
2009 (119)	Human	In vivo – nude mice	–	–	–	Grafting of adipose tissue stimulates neosynthesis of collagen increasing dermal thickness
2008 (120)	Human	In vivo – humans	–	–	–	New collagen deposition, local hypervascularity and dermal hyperplasia
2007 (125)	Human	Both – nude mice	Collagen	–	Fibroblast proliferation	ADSC enhance collagen synthesis by HDFs and activate HDF proliferation & migration
2007 (121)	Human	In vivo – humans	–	–	–	Improvement of ultrastructure, neovessel formation and systematic improvement

ADM, acellular dermal matrix; BFGF, basic fibroblast growth factor; Co-CS-HA, collagen-chondroitin sulfate-hyaluronic acid; EGF, epidermal growth factor; GFP, 6-green fluorescent protein; HDF, human dermal fibroblast; HGF, hepatocyte growth factor.

have been evaluated, both natural and synthetic, including silk fibroin-chitosan (124), collagen (125) collagen-chondroitin sulfate-hyaluronic acid (126) and acellular dermal matrix (126). While there is no consensus on the most appropriate material to construct a scaffold, key features can be identified as desirable between each of the studies. In addition to conforming to the wound shape, they should provide sufficient stability to allow cells to proliferate and mature while resorbing in sufficient time so as to not interfere with wound maturation (127). Scaffolds can create a wound–stem cell interface to control the interaction and the rate at which the cells are introduced to the wound (112). Scaffolds can affect cell adhesion, with some studies suggesting that a type 1 collagen sponge is ideally suited because of its porous structure (114). It is also important that the collagen degrades to allow for the migration, proliferation and differentiation of cells (71). A mixture of fibroblasts and ADSCs can improve the epidermal morphogenesis of tissue-engineered skin (128). ADSCs have the ability to grow on porous biomaterials and differentiate into fibrovascular, endothelial and epithelial tissues (124). ADSCs also enhance skin regeneration through their secretory effects on dermal fibroblasts (125) and keratinocytes (129).

Conclusion

The clinical applications of ADSCs are broadly ranged; the ease of cell harvest and high yield with minimal donor-site morbidity make them an ideal cell source. Additionally, the multi-lineage potential of these cells demonstrates the significant opportunities they present within the field of tissue engineering, with studies successfully demonstrating the ability to produce a range of tissue types. Future challenges lie within the application of these technologies and the steps required to bridge the transitional gap that currently exists. In particular, the mechanism of action of ADSCs, their interactions with extracellular matrix microenvironments and long-term fate require further clarification. Additionally, randomised trials demonstrating the continued safety of transplanted ADSCs as well as the efficacy of cellular therapies in comparison to commonly applied conventional techniques are fundamental.

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